J. Clin. Chem. Clin. Biochem.
Vol. 21, 1983, pp. 11–17

Metabolism and Proliferation of Cultured Fibroblasts from Specimens of Human Palmar Fascia and *Dupuytren*'s Contracture[1])

The pathobiochemistry of connective tissue proliferation, II[2])

By *A. Delbrück* and *H. Schröder*

Institut für Klinische Chemie II, Zentrum Laboratoriumsmedizin der Medizinischen Hochschule Hannover

(Received March 20/August 3, 1982)

Summary: Cell Cultures from 11 *Dupuytren*'s contracture and 6 normal palmar fascia specimens were established. The rates of sulphated glycosaminoglycan, collagen and DNA synthesis by means of incorporation of labelled precursors ([^{35}S]sulphate, [^{3}H]proline, [^{3}]thymidine) as well as the growth characteristics of the cell lines of both healthy and diseased were compared. The incorporation rates of [^{35}S]sulphate and [^{3}H]proline were found to be significantly higher in *Dupuytren* than in healthy palmar fascia-deriving cell lines. In contrast, no differences in cell growth or DNA synthesis could be demonstrated. The abnormal capacity to synthesize sulphated glycosaminoglycans and collagen is attributed to a permanent modulation of cell characteristics which can be propagated into cell culture.

Stoffwechsel und Proliferation von Fibroblasten aus menschlicher Palmarfaszie und Dupuytren'schem Kontrakturgewebe in Zellkultur

Zur Pathobiochemie der Bindegewebsproliferation, 2. Mitteilung

Zusammenfassung: Kulturen von Zellen aus 11 Proben von *Dupuytren*'scher Kontraktur und 6 normalen Palmarfaszien wurden eingerichtet. Die Syntheseraten sulfatierter Glycosaminoglycane, von Kollagen und DNA wurden anhand der Einbauraten markierter Präcursoren ([^{35}S]Sulfat, [^{3}H]Prolin, [^{3}H]Thymidin), bestimmt und die Wachstumseigenschaften der Zell-Linien erkrankter und gesunder Gewebsproben verglichen. Die Einbauraten von [^{35}S]Sulfat und [^{3}H]Prolin waren in den Zellen von *Dupuytren*-Gewebe signifikant höher als in den Zell-Linien von Normalgewebe, während sich keine Unterschiede für die DNA-Synthese und das Wachstumsverhalten ergaben. Die Unterschiede in den Syntheseraten werden als Ausdruck einer permanenten Modulation der Zelleigenschaften interpretiert, die vom Gewebe in die Zellkultur übertragen werden können.

Introduction

Uncontrolled tissue proliferation characterizes chronic diseases like rheumatoid arthritis, *Dupuytren*'s diseases or liver cirrhosis. Augmentation of cells and enhanced synthesis of extracellular matrix components occur simultaneously with the breakdown of the extracellular structure in the afflicted tissues. The pathomechanism that maintains and perpetuates the diseased state is so far unknown and may be based on disturbed cellular metabolism or on the effect of a variety of factors emerging from endogenous or exogenous processes occuring in the pathogenetic sequence of the respective diseases. These factors are thought to be active on the cells as well as on the extracellular matrix of connective tissues. To obtain more insight into the pathologic

[1]) With the support of the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 54)
[2]) I = l. c. (20)

0340-076X/83/0021-0011$02.00

reaction mechanism on the cellular level, cell cultures of fibroblasts from normal and uncontrolled proliferating tissues were established. *Dupuytren*'s contracture tissue was chosen, because this tissue shows an uncontrolled proliferation whose aetiology is so far unknown, but has been discussed in respect of similar pathologic processes underlying other chronic proliferative diseases, like heredety (1, 2), sex disposition (1), chronic inflammation and autoimmune reaction (3–6) or benign tumours (7). Biochemical investigations of *Dupuytren*'s contracture tissues revealed increased glycosaminoglycan and collagen contents (8–12) as well as the appearance of type III collagen (13–17). The aim of the present study was to answer the question whether fibroblasts transferred from *Dupuytren*'s contracture into cell culture exhibit corresponding differences, with respect to cell growth and rates of synthesis of fibre protein and ground substances, compared with cells from normal human palmar fascia.

Materials and Methods

Biopsy specimens from 11 patients (aged 30 to 70 years) suffering from *Dupuytren*'s contracture of the hand and 6 specimens from autopsy cases (aged 31 to 80 years, 24 hours post mortem) were taken under sterile conditions for cell culture. The specimens were freed from adjacent tissue and blood and small segments of specimens from 2 to 2 mm were incubated in Falcon flasks with minimum essential medium (*Earl*'s balanced salt solution with 10% foetal calf serum) at 37 °C under 95% air/5% CO_2. The growth medium was changed every 3rd day. After 3 to 4 weeks a cell layer had formed. Cells were harvested by means of trypsin treatment (trypsin 125 U/l [Serva, Heidelberg, Germany] EDTA 0.6 mol/l) and seeded into Falcon flasks as above for subpassages. After the second passage the cells were frozen in small portions and kept at −80 °C. From this pool cells were available for incorporation experiments which were performed in quadruplicate at the 2nd to the 5th subculture stage.

Determination of DNA-, glycoaminoglycan- and collagensynthesis in vitro

Cell culture conditions were as described above with the exception that the foetal calf serum concentration was 3% instead of 10%, and a sulphate-free medium was used for [^{35}S]sulphate incorporation experiments. For [^{3}H]proline incorporation assay, ascorbate was added to the medium to a final concentration of 0.3 mmol/l. Pulse time 48 hours.

The concentration of labelled precursors:
[6−^{3}H]thymidine: 63 MBq/l
(185 GBq/mmol thymidine)
[6−^{3}H]uridine: 37 MBq/l
(580 GBq/mmol uridine)
[3.4−^{3}H]*L*-proline: 63 MBq/ml
(2035 GBq/mmol proline)
[^{35}S]sulphate: 185 TBq/l
(185 KBq/mg sulphur)
(Amersham-Buchler, Braunschweig, Germany)

The incorporation rates are espressed in Bq/10^6 cells.

Cell count

Cells were harvested by pronase treatment (1 g/l phosphate buffered salt solution, pH 8, Serva, Heidelberg, Germany), washed and resuspended in an appropriate volume of buffered salt solution (TOA Cellkit 7). The cell numbers were determined in quadruplicate by means of a Sysmex Cell Counter LC 110 (TOA Medical Electronics CO, Ltd., Kobe, Japan).

DNA assay

The *Burton* procedure (18) was modified by introducing proteolysis by papain before the DNA precipitation to remove proteins from the ultrasonic irradiated cell suspension. Following DNA precipitation by perchloric acid (final concentration 1 mol/l) and centrifugation at 2400 *g* for 15 minutes, the pellet was hydrolysed in 0.5 mol/l perchloric acid for 20 minutes at 70 °C. Photometry was performed with diphenylamine reagent at 600 nm in a Beckman Spectrophotometer 25 (Beckman Instruments GmbH, Munich, Germany).

Hydroxyproline determinations were carried out by the *Stegemann* method (19).

Determination of the incorporation rate of [^{3}H]proline activity into collagen [^{3}H]hydroxyproline

The culture medium was decanted and stored at 4 °C. The cells were freed from the culture well by pronase followed by mild alkaline treatment with 0.1 mol/l NaOH to improve protein removal from the cell surfaces. After neutralization with hydrochloric acid the cell suspension was treated by ultrasonic irradiation for 15 minutes. For the [^{3}H]proline activity incorporation assay the culture medium and the particulate cell fraction were recombined and exhaustively dialysed against phosphate buffered (pH 7.2) 0.16 mol/l sodium chloride solution. After hydrolysis of the samples in 6 mol/l hydrochloric acid for 24 hours at 100 °C the hydrolysates were evaporated (Büchi Rotation Evaporator, Büchi, Flawil, Switzerland) to dryness, redissolved in 0.8 ml 1 mol/l hydrochloric acid and centrifuged for 5 minutes at 18000 *g* (Minifuge 2, Heraeus-Christ, Osterode, Germany) to clear the solutions. For column chromatography carrier proline and hydroxyproline were added to the supernatants in final concentrations of 0.8 mmol/l and 3 mmol/l, respectively. Column chromatography was done on Dowex 50 WX 8, 200–400 mesh (column bed 10 × 280 mm and 22 ml volume, ascending elution with 1 mol/l hydrochloric acid). A constant elution flow of 0.6 ml/minute was provided by means of a Technicon Autoanalyzer proportioning pump (Technicon GmbH, Frankfurt/Main, Germany) equipped with precalibrated pumping tubing 0.040 mm i. d. for 10 parallel runs. The constancy of the flow rate meant that it was necessary to monitor the effluent of only one of the 10 columns for radioactivity. The peak positions were identified by parallel determinations of radioactivity and chemical analysis of hydroxyproline in the effluent fractions. Aliquots of the respective fractions were taken for radioactivity measurements in the Packard Tricarb Liquid Scintillation Counter 2660 using Packard Scintillator 299 (Packard Instruments, Frankfurt/Main, Germany).

Determinations of the incorporation rate of [^{3}H]thymidine

The culture medium was decanted and the cell monolayer treated with methanol. The cell monolayer was washed three times with ice-cold phosphate buffered 0.16 mol/l sodium chloride solution, pH 7.2, followed by two washings with ice-cold 0.6 mol/l trichloracetic acid, finally washed twice with ether: ethanol 1:5, and dried in the open air. Each sample was resuspended in 2 ml 0.5 mol/l sodium hydroxide and kept for 24 hours in a moist chamber at 37 °C. Subsequently the samples were neutralized with hydrochloric acid and aliquots were taken for measurement of radioactivity, using a Packard Scintillator 299.

Incorporation of [^{35}S]sulphate

The samples were prepared in the same way as for [^{3}H]proline incorporation with the exception that dialysis was initially performed against 0.1 mol/l ammonium sulphate pH 7.2, followed by exhaustive dialysis against phosphate-buffered sodium chloride solution 0.16 mmol/l, pH 7.2. Aliquots of the dialysed samples are taken for measurement of radioactivity (Packard Scintillator 299, Tricarb Counter).

Statistics

The significance of the differences of data obtained from cells of *Dupuytren*'s contracture and normal palmar fascia was tested by the T-Test.

Results

Suitable conditions were determined for the culture of fibroblasts from both *Dupuytren*'s contracture and normal palmar fascia. They meet the requirements for reproducible experiments on the metabolism of connective tissue cells in vitro. The *Burton* method (18) of DNA determination was improved by employing enzymatic proteolysis to disintegrate tissues or cells layers and to remove proteins before the DNA precipitation. Extensive dialysis of samples against appropriate buffered salt solutions proved to be the most effective procedure for complete removal of excess label from the incubation mixture. The chromatographic separation of hydroxyproline and proline on Dowex 50 WX8 resulted in a clear-cut separation of two distinct peaks. Since the flow rate was controlled and constant, it was necessary to monitor effluent radioactivity in only one out of ten parallel run columns. The recovery for both compounds was about 100% (tab. 1). The precision of the methods employed in this study are given in table 1. The overall precision of the incorporation experiments (4 parallel runs of cells from the same cell line) was less than 12% (for detail see tab. 1). However, this applies only to such experiments as were conducted simultaneously using the same lot of labelled precursors, the same test solutions, and simultaneous incubation of the culture wells in the same incubator. The biological variation between the cell lines of the same origin was considerably higher than the experimental variation within parallel runs of cells from an individual cell line (see tab. 2), even comparing incorporation rates of precursors at the same cell density level of the cultures.

Tab. 1. Reliability of methods. Coefficient of variation within series of cell cultures from one individual fibroblast line (n = 4, for [^{3}H]thymidine n = 8, for chromatography n = 10).

Method	CV (%)
Cell count (TOA)	6
DNA (modified *Burton*)	10
Incorporation of	
– [^{3}H]hydroxyproline	12
– [^{3}H]proline	12
– [^{35}S]sulphate	10
– [^{3}H]thymidine	10
[^{3}H]proline/[^{3}H]hydroxyproline chromatography, recovery	100 ± 5*)

*) Refers to the chromatography procedure of hydrolysates, only.

Tab. 1. Experimental and biological variation of labeled precursor incorporation experiments in 4 lines of *Dupuytren*'s contracture-derived cultured fibroblasts. $\bar{x}$ mean of four parallel runs of one individual cell line (D-1, D-2, D-3, D-6) and of data from the different cell lines, respectively. All experiments were done under identical conditions at approximately the same cell density level. For details see methods.

	Methodical variation within individual cell lines								Biological variation between cell lines	
	D 1 (n = 4)		D 2 (n = 4)		D 3 (n = 4)		D 6 (n = 4)		(n = 4)	
	$\bar{x}$, s	CV	$\bar{x}$, s	CV	$\bar{x}$, s	CV	$\bar{x}$, s	CV	$\bar{x}$, s	CV
Cell density 10^3 cells/cm^2	68 ± 8.7		71 ± 1.5		56 ± 4.1		60 ± 1.7			
[^{3}H]thymidine incorporation Bq/10^6 cells	6.0 ± 0.48	8.1%	6.83 ± 0.28	4.2%	6.78 ± 0.68	10.1%	2.65 ± 0.02	1.4%	5.57 ± 1.97	35%
[^{35}S]sulphate incorporation Bq/10^6 cells	9.27 ± 0.85	9.2%	3.38 ± 0.25	7.4%	4.57 ± 0.22	4.7%	3.67 ± 0.01	0.3%	5.22 ± 2.73	52%
[^{3}H]hydroxyproline Bq/10^6 cells	2.13 ± 0.27	12.2%	1.7 ± 0.09	5.3%	2.4 ± 0.27	11.1%	1.8 ± 0.23	13%	2.0 ± 0.32	16%

Furthermore the DNA contents of the cell culture proved to be unsuitable for reference purposes, because the cell DNA concentration decreased with increasing cell density (fig. 1). The results of 11 experiments comparing the incorporation of [^{3}H]thymidine, [^{35}S]sulphate and [^{3}H]proline (hydroxyproline) of cultured cells from *Dupuytren*'s contracture and normal palmar fascia are listed in table 3. The data of the individual runs are derived from simultaneous cultures of approximately the same cell density from *Dupuytren* and normal fibroblasts. While the [^{3}H]thymidine incorporation rates showed only slight differences between *Dupuytren*'s contracture cells and normal palmar fascia (with the exception of 0205/1), in each case the incorporation rate of the glycosaminoglycan and the collagen precursors of *Dupuytren* cells significantly exceeded those of the respective palmar fascia cells. On average, the

Tab. 3. Metabolic activity of cultured fibroblasts from *Dupuytren's* contracture in comparison with normal palmar fascia fibroblasts. Each single experiment was done simultaneously for fascia and *Dupuytren* cells under the same experimental conditions and at the approximate same cell density level in quadruplicate. Initial cell density of cultures 15000/cm^2. Incorporation experiments were carried out in confluent cell state.
D = cells from *Dupuytren*'s contracture
F = cells from palmar fascia
$\bar{x}$ = mean of four parallel runs for each cell line (n = 4)
p = level of significance between the labeled precursor incorporation rates of *Dupuytren* and the respective palmar fascia cells (T-test)
n. d. = not determined
( ) = Age of cell donors

Experiment Nr.	Paired cell lines	Cell density 10^3/cm^2 $\bar{x}$, s	[^{3}H]Thymidine Bq/10^6 cells $\bar{x}$, s	[^{35}S]Sulphate Bq/10^6 cells $\bar{x}$, s	p	[^{3}H]Hydroxyproline Bq/10^6 cells $\bar{x}$, s	p
3105/1	D-11(50)	47 ± 2.7	n. d.	18.9 ± 0.12	0.0001	1.03 ± 0.18	0.05
	F-4(80)	38 ± 1.9		10.2 ± 0.25		0.43 ± 0.043	
0205/1	D- 9(66)	42 ± 1.2	14.9 ± 0.33	12.4 ± 0.27	0.001	n. d.	0.001
	F- 3(31)	55 ± 1.7	9.85 ± 0.3	8.37 ± 0.52			
0205/2	D-14(56)	40 ± 1.6	10.5 ± 0.11	11.5 ± 0.27	0.001	n. d.	0.001
	F- 3(31)	55 ± 1.7	9.85 ± 0.3	8.37 ± 0.52			
0205/3	D- 8(33)	54 ± 0.9	7.78 ± 0.52	13.8 ± 0.75	0.0001	1.58 ± 0.22	0.001
	F- 3(31)	55 ± 1.5	9.85 ± 0.3	8.37 ± 0.52		0.70 ± 0.08	
1603/1	D- 3(52)	69 ± 1.7	7.25 ± 0.37	6.33 ± 0.08	0.001	n. d.	
	F- 2(46)	61 ± 1.3	11.4 ± 0.22	4.77 ± 0.25			
3105/2	D- 5(70)	72 ± 3.5	1.33 ± 0.07	14.8 ± 0.53	0.001	0.97 ± 0.037	0.0001
	F- 4(80)	38 ± 1.9	1.18 ± 0.02	10.2 ± 0.25		0.43 ± 0.038	
1603/2	D- 1(61)	77 ± 2.1	9.18 ± 0.12	12.2 ± 2.1	0.0001	n. d.	
	F- 2(46)	61 ± 1.3	11.5 ± 0.22	4.78 ± 0.25			
2112	D-21(29)	79 ± 1.9	n. d.	9.32 ± 0.08	0.0001	2.38 ± 0.1	0.0001
	F-10(60)	110 ± 2.6		5.95 ± 0.32		1.23 ± 0.027	
1805	D- 8(33)	105 ± 11	2.55 ± 0.08	8.28 ± 0.03	0.0001	n. d.	
	F- 3(31)	103 ± 8.7	3.25 ± 0.15	5.42 ± 0.2			
2805	D- 9(66)	107 ± 5.4	2.28 ± 0.08	19.9 ± 0.72	0.0001	1.32 ± 0.002	0.0001
	F- 6(36)	116 ± 4.9	2.37 ± 0.13	10.9 ± 0.33		0.517 ± 0.002	
2610	D-19(59)	125 ± 4.1	n. d.	8.82 ± 0.28	0.001	0.95 ± 0.028	0.01
	F- 7(75)	109 ± 6.0		6.97 ± 0.17		0.97 ± 0.027	

[^{35}S]sulphate incorporation rate was 1.6 times, the [^{3}H]hydroxyproline rate 2.4 times greater (fig. 2). This was also true for experiments with the same cell lines at different cell densities (see 0205/3 and 1805). These differences were also shown between an individual palmar fascia cell line and 3 and 2 different *Dupuytren* cell lines, respectively, (0205/1, 0205/2, 0205/3 or 1603/1, 1603/2). In accordance with the lack of difference in [^{3}H]thymidine incorporation rates the growth characteristics of cells from palmar fascia and from *Dupuytren*-derived cells were identical (fig. 3); the DNA contents of the respective cells of both groups did not differ (fig. 1) and declined with increasing cell densities of the cultures. The differences in metabolic activities and the characteristics of cell growth were constant from the 2nd to the 5th subculture stage.

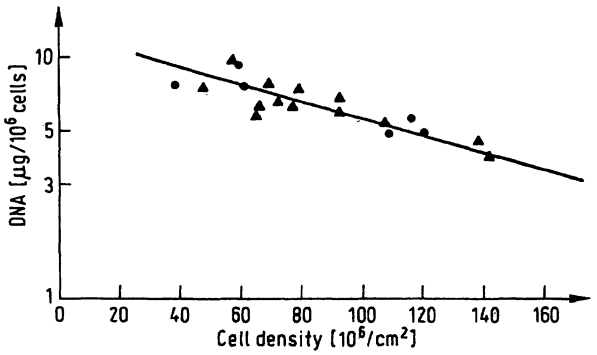

Fig. 1. DNA contents of cells derived from *Dupuytren's* contracture (▲, n = 13) and normal human palmar fascia (○, n = 6) in dependence on the cell density of the culture. DNA assay modified according to *Burton* (18).

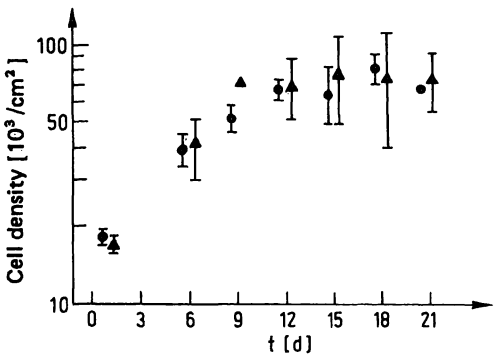

Fig. 3. Cell growth of lines from *Dupuytren's* contracture (▲) and normal human palmar fascia (○) Cell count in quadruplicate (TOA Sysmex Cellcounter). Each figure represents the mean of 3 different cell lines, the bars indicate the standard deviation.

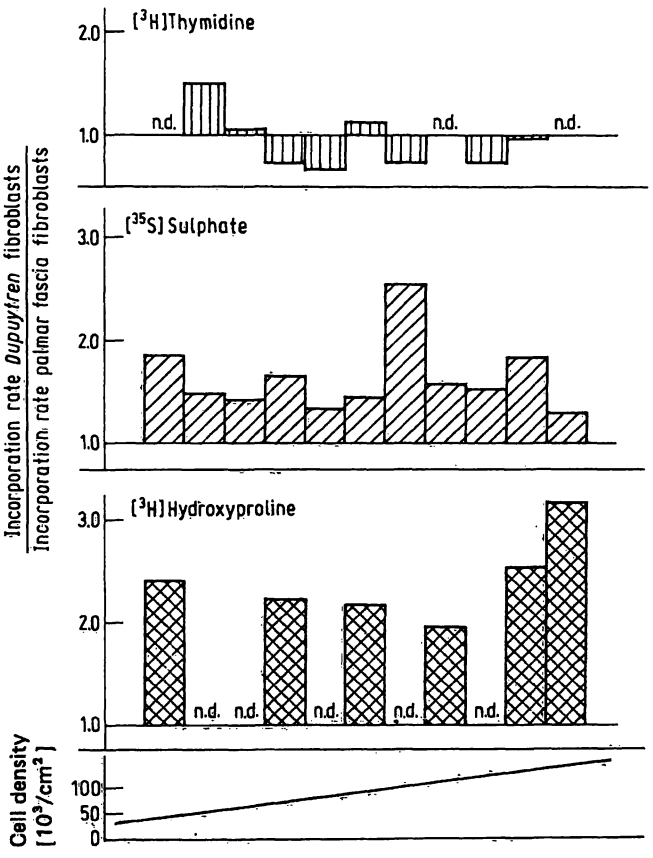

Fig. 2. Incorporation of labeled precursors into DNA, glycosaminoglycans and collagen by cultured fibroblasts from *Dupuytren's* contracture and normal human palmar fascia. Determinations in quadruplicate cultures, *Dupuytren* and palmar fascia lines are matched according to the cell density. The incorporation rates of palmar fascia are set at 1.0 for detailed data see table 2
n. d. = not determined

Discussion

The data presented demonstrate significantly higher incorporation rates of labelled precursors into glycosaminoglycans and collagen by cultured fibroblasts from *Dupuytren's* contracture, compared with cells from normal palmar fascia. The reliability of the methods applied depends on absolutely identical test conditions, which can only be achieved if the parallel determinations on *Dupuytren* and palmar fascia cells are run simultaneously to minimise experimental variations. Although the test system comprises parallel cell cultures and complex isolation steps of the reaction products, the overall coefficients of variation of less than 12% for the methods used meet the experimental demands. In the interpretation of the data, the biological variations between the single cell lines have to be taken into consideration. Reproducible results could not be expected unless the cell cultures under investigation were matched according to their cell density; this is because, as shown previously (20), the metabolic activities of the cells depend on the density of cell monolayers. Cells are the metabolic unit and the site of synthesis of the extracellular components of connective tissue. Therefore the cell was chosen as a reference for the observed metabolic activities of glycosaminoglycan-, collagen-, and DNA-synthesis. The DNA content of cell cul-

tures depends on the growth state of the individual cell line (fig. 3) and varies like the protein content or enzyme activities (20). Therefore, neither the protein nor DNA content seems suitable as a reference for metabolic activities. [^{35}S]sulphate incorporation into the macromolecular components of the cell layer and the culture medium can be regarded as an indicator for the synthesis of sulphated glycosaminoglycans. [^{3}H]proline, as precursor of the collagen hydroxyproline, can be assumed to be almost a specific parameter for collagen synthesis in cell culture. The same is true for [^{3}H]thymidine for DNA synthesis. This is in accordance with the observations of *Tessari & Parini* (10), *Lagier & Exer* (11), *Carr* (8), *Viljanto* et al. (12) and *Hunter* et al. (9) who demonstrated elevated collagen contents in tissue specimens from *Dupuytren*'s contracture. In addition, these authors published data on the hexosamine and the total glycosaminoglycan concentration in *Dupuytren*'s contracture which paralleled the changes in the collagen contents. Both the in vivo experiments and the in vivo findings suggest that some abnormal metabolic characteristics of cells from diseased proliferating tissues can be carried forward from the affected tissues in vivo into in vitro cell cultures, and that they propagate from one generation of cells to the next. This phenomenon leads to the conclusion that the connective tissue cell in vivo has undergone fundamental changes in metabolic control which have finally resulted in impaired composition of the extracellular structure of the afflicted palmar fascia in *Dupuytren*. The occurrence of such variation at the cellular level does not seem to be restricted to *Dupuytren*'s contracture but applies more or less to a variety of connective tissue diseases, although they may expressed differently. Similar observations on glycosaminoglycan synthesis have been published by *Castor* et al. (21–23) for cultured synovial cells from patients suffering from rheumatoid arthritis. *Diegelmann* et al. (24) reported a higher collagen synthesis per fibroblast in keloid-derived cells compared with cells from normal skin. Cultured cells from human cirrhotic liver biopsies are characterized by their "myofibroblast"-like morphology (25), which mimics smooth muscle cells from vessels walls in culture; such cells have also been demonstrated in *Dupuytren* tissue, (14). These cells are found to grow out from fibrotic liver explants only, and they show elevated synthesis rates of glycosaminoglycans and collagens (26) in vitro, corresponding to an increased collagen and glycosaminoglycan synthesis in vivo (27–29). Finally *Hauss* and coworkers (30, 31) succeeded in growing arterial wall smooth muscle cells out of explants from atherosclerotic rat aorta, which propagated an elevated cell growth through 2 to 6 subcultures. All these published data support the conclusion that cells from chronic proliferating tissues have an automonous capacity to synthesise collagen and glycosaminoglycans at an increased level in vitro and likewise in vivo. Controversial results are reported concerning the growth rate of cultured cells from the various diseased tissues. While human cells cultured from *Dupuytren* (fig. 3), keloid, and normal scar (32) exhibit no significant differences, the growth rates of cells from the aorta wall of arteriosclerotic rats exceeds that of cells from normal rat aorta (30). The lack of increased cell growth in *Dupuytren* cells is in good agreement with the almost unchanged [^{3}H]thymidine incorporation rates of *Dupuytren* derived cells in vitro, which are within the biological variation between cell lines (fig. 2, tabs. 2, 3). There is no evidence so far as to the mechanism leading to the modulation of the cells in pathological tissue proliferation. Besides alterations in the genetic control, one has to consider the findings of *Castor* (23) who was able to demonstrate elevated levels of the connective tissue activation peptide in cultured rheumatoid synovial cover cells. This would imply production of growth and metabolism regulating factors by the cells themselves. The appearance of type III collagen in *Dupuytren*'s contracture (13–17), alterations of collagen and glycosaminoglycan distribution in various fibrotic tissues like liver cirrhosis (25–29, 33–35) or atherosclerosis (30, 31, 36), and corresponding findings in cultured cells from the respective tissues suggest an impaired genetic control. Further studies should reveal detailed data on the glycosaminoglycan pattern of *Dupuytren*'s contracture in vivo and in vitro, their relation to the collagen type composition and the possible role of endogeneous and exogeneous cell growth and/or metabolism-stimulating factors in the aetiology of this disease.

Acknowledgements

The authors are indebted to colleagues at the Clinic for Hand and Reconstruction Surgery, Hannover Medical School for their assistance in obtaining biopsy specimens during operation, to colleagues at the Institute of Pathology, Hannover Medical School, for their help in collecting the autopsy specimens, and to colleagues in the Department of Biometrics, Hannover Medical School for statistical advice. The authors express their gratitude to Miss *Ursula Pöhls* for skilled technical assistance.

W DE G

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Insulin
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Proceedings of the Second International Insulin Symposium, Aachen, Germany, September 4–7, 1979

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ISBN 3 11 008613 1

This book groups together under one single cover antagonists for those hormones where antagonism has been documented specifically and with a certain degree of certitude. The major emphasis has been delineation of anti-hormone activity at the level of the hormone receptor but other aspects, such as antibody mediated antagonism and inhibition of synthesis, have been included to indicate other possible levels of inhibition of hormone activity. Clinical aspects, too, have been covered where they were documented with certitude.

It is felt that the book represents a major new reference source for years to come. Scientists, medical academicians, and advanced graduate students may use the book as a departing point for further pursuit of their own field. Involved research workers will find the volume of much interest since it provides data not published elsewhere. The book may also be used as a text volume to indicate the diversity and the wealth of information on the subject of hormone antagonism both in the basic research and in clinical medicine. Photo-offset method of publication assures expediency before specialized articles obsolete novelty.

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Prof. Dr. med. A. Delbrück
Institut für Klinische Chemie II
im Zentrum für Laboratoriumsmedizin
der Medizinischen Hochschule Hannover
Podbielskistr. 380
D-3000 Hannover 51